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The Effect of Methandrostenolone on the Uptake of Radio-phosphorus in Rat Molars

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THE EFFECT OF METHANDROSTENOLONE ON THE UPTAKE OF RADIO-PHOSPHORUS IN RAT MOLARS

BY

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

There is probably no disease that is more common to every man, woman, and child than dental caries. Although caries is not a serious disease in terms of fatalities, it remains as one of modern medicines most common uncurable diseases and one of modern man's most consistent medical expenses. Dental caries is not only an infectious disease (1), but it is also directly effected by genetic, dietary, and environmental factors. To find a cure for dental caries is obviously a very complex problem, and the contribution of each of these factors must be understood before a solution can be evolved. Of particular interest is the relation of protein to the structure of the tooth and the decay process.

Prior to eruption of the tooth, fibrous protein is mineralized (2,3) and enveloped by inorganic hydroxyapatite to form the enamel of the tooth. During this time, blood serum has much to do with the growth of teeth. Sobel and Hanok (4) showed that the composition of the diet is reflected in the blood serum and that the composition of teeth is related to that of serum. After eruption, oral fluids and environmental conditions also affect tooth composition. It has been demonstrated (5,6,7,8) that there is a mechanism for transport of phosphate from saliva to dentin by way of the enamel as well as a reverse mechanism from dentin to enamel. Bevelander and Amler (7) found that phosphorus atoms of blood plasma exchange with those of teeth. The rate of exchange is greater for incisors and unerupted teeth than for older and mature teeth. Wynn (9), McClure (10), Sobel (11), and their

colleagues have demonstrated that sodium and calcium phosphates have an inhibitory effect on dental caries in the rat. However, McClure (10) found that it does not appear likely that the primary effect of sodium phosphate involves a systemic utilization of the phosphate supplement.

Keyes (1,12,13,14) has shown caries to be an infectious and transmissible disease, and others (1,3,15) have demonstrated bacterial penetration and proteolysis in enamel and dentin. Following bacterial proteolysis of tooth protein, acid decalcification occurs as a second phase in the process of tooth destruction, i.e., the formation of caries.

Because protein is an essential structural component of teeth and has an important role in the production of caries, the relation of protein anabolic agents to dental caries was of interest. Protein anabolic agents reverse negative nitrogen balance by promoting retention of nitrogen and synthesis of tissue protein. Androgens, such as testosterone, elicit this type of biological response. Muhler and Shafer (16) found that testosterone significantly increased dental caries in the intact rat. Since testosterone results in both anabolic and androgenic activity, it was considered worthwhile to study the effect of a drug having little or no androgenicity but anabolic activity equal to or greater than that of testosterone. The results of such a study might indicate whether the androgenic or the anabolic effect or both are responsible for the increase in caries. Shaw and Bailey (17) used norethandrolone* for this purpose. Norethandrolone is a synthetic steroid anabolic agent that possesses anabolic activity equal to testosterone

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*Nilevar - G. D. Searle and Co., Chicago, Illinois.

but has a low androgenic effect. Their results showed that norethandrolone was cariogenic under the experimental conditions used by them. In another study Shaw and Bailey (18) found no difference in radio-phosphorus uptake in rat molars, compared with controls, upon administration of norethandrolone.

During Shaw and Bailey's experimentation, Ciba Research Laboratories discovered and marketed a new synthetic anabolic drug called methandrostenolone*. Laboratory evaluations of methandrostenolone (19) showed that it had an androgenic effect 1/150 to 1/250 that of testosterone propionate, 1/70 to 1/30 that of norethandrolone, and 1/70 that of methyltestosterone. The oral anabolic to androgenic ratio was 4.5 times as effective as methyltestosterone, while norethandrolone was 3.0 times as effective as methyltestosterone. Not only is methandrostenolone a potent anabolic agent, it is used in osteoporosis to relieve pain and to encourage calcium utilization. Since calcium is an integral part of hydroxyapatite, this property might also be beneficial in a study of dental caries.

Because of the favorable anabolic to androgenic ratio of methandrostenolone, it was decided to study the effect administration of this drug would have, if any, on phosphorus uptake and exchange in rat molars by using radio-phosphorus. The results of this work could thus be compared to those found by Shaw and Bailey upon administration of norethandrolone. This comparison might show how androgenicity effects the radio-phosphorus uptake in the molars of the rat.

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*Dianabol - Ciba, Summit, New Jersey.

Since Shaw and Bailey were interested in the cariogenic activity of norethandrolone, as well as its effect on radio-phosphorus uptake in the molars of the rat, they used caries-test diets. In an effort to duplicate their experimental conditions, the same carries-test diets were used. This included caries-test diets in which the phosphorus content had been increased by the addition of disodium phosphate to obtain a comparison with a known cariostatic agent.

REVIEW OF LITERATURE

A basic understanding of the processes of caries formation is necessary for a study of the effect that a protein anabolic agent has on mineral uptake in teeth, and the relation of this to dental caries. For this reason, a general survey of present day theories concerning the etiology of this disease is presented.

Dental caries is a complex disease probably having no one causative factor. That bacterial action is essential has been definitely shown by Keyes and his co-workers (1). However, the presence of cariogenic bacteria alone is not enough to produce caries in teeth. The metabolic makeup of the host as affected by genetic, dietary, and environmental factors is also of great importance. As in all disease states, the causative factors must all be present in proper quantitative proportions for the biological effect to be initiated. Of first importance is the bacterial factors of dental caries.

Following demonstration of bacterial penetration in caries of the enamel (1,3,15), it was recognized that the disease is not entirely one of decalcification. Rather, tooth destruction is now considered to be a two phase process in which both acid decalcification and bacterial proteolysis play a part. On this basis, a working hypothesis has evolved that, following infection with species-specific, causative organisms and establishment of a lesion in tooth enamel, caries is a degradation of the organic matrix resulting from the enzymatic action of microorganisms, which is in turn followed by a physical disintegration of the inorganic salts.

Keyes (1,12,13,14) has shown in both hamsters and Osborne-Mendel rats that dental caries appears to be an infectious and transmissible disease. In a study of restricted caries, i.e., caries whose penetration is slowly progressive and is evidenced by the appearance of a brown-stained spot in the intact enamel surface of the tooth, Frisbie and Muckalls (3) found that spherical microorganisms are first to invade the dentin. These spherical microorganisms cause degradation of the enamel matrix and the dentin with degradation occurring more rapidly in the dentin due to a greater content of organic material. During the final stages of degradation, the spherical organisms are dominated by threadlike forms of organisms. Following degradation of the enamel matrix, the enamel is left vulnerable to acid decalcification and removal of the inorganic salts.

In view of these theories of dental decay, the mineral and protein content of teeth is directly related to the formation of caries. Sobel, Shaw, Hanok and Nobel (20) consider that the inorganic fraction of teeth (or mineral crystalline core of hydroxyapatite) is surrounded by a tightly bound amorphous adsorption layer. The crystalline core of hydroxyapatite can vary from $Ca_6(PO_4)_6OH_2^{8-}$ to $Ca_{14}(PO_4)_6OH_2^{8+}$. The adsorbed outer shell contains anions such as phosphate, carbonate, citrate, and flouride; and cations such as calcium, sodium, magnesium, lead and strontium.

Therefore, the composition of the adsorption layer determines the initial solubility of the tooth mineral. Citrate and carbonate present in relatively large amounts increase tooth solubility, while fluoride would decrease solubility. Sobel, et al. (20), varified that

low carbonate teeth are less caries-susceptible than high carbonate teeth in the cotton rat.

Considering the growth of enamel (2,3), it is agreed that a definite orientation of fibrous protein exists in the pre-enamel matrix which subsequently undergoes a progressive mineralization. It was found that the beginning of calcification was confined to this fibrous area and that the fibrous protein became enveloped by the inorganic hydroxyapatite crystals which grew along the fibrils. This led to the realization that there is a complete organic matrix within the enamel of the tooth.

Volker and Sognnaes (21) found that enamel has approximately 1-2 per cent organic material. The principal mineral constituents are calcium and phosphorus which make up 36 per cent and 17 per cent of the dry weight, respectively. They also found that dentin contains approximately 25 per cent organic material while the total dry weights of calcium and phosphorus were 26 per cent and 12.5 per cent, respectively.

Prior to eruption of the tooth, the internal body fluids and "local factors" influence its mineral composition. After eruption, the oral fluids also exert an influence. The oral environment particularly affects the outermost surface of the tooth, but Pincus (6) showed that phosphate can pass through odontoblasts, dentin, and enamel of recently removed human teeth in which the tooth pulp is still respiring. This permeability of enamel may be related to the heterogeneity of enamel.

Barnum and Armstrong (5) also established the existance of mechanisms for transport of phosphate from saliva to dentin by way of the enamel. The transport of phosphate from blood to the enamel by way

of the dentin was demonstrated with radiophosphorus. Their experiments demonstrated that the radiophosphorus acquired by the enamel of fully formed teeth was derived in part from the saliva, but apparently in largest amount from the blood. The enamel phosphorus of molar teeth of rats was also shown not to be completely renewed by exchange in 116 days.

Bevelander and Amler (7) also found that phosphorus atoms of the blood plasma exchange with those of the teeth. They showed that actively growing teeth, such as rat incisors, have a higher rate of absorption than the mature teeth of the rat and other animals. In line with these findings, Volker and Ginn (22) found that the radioactive phosphorus metabolism of calcified portions of unerupted enamel and dentin is of a much greater magnitude than the same tissues of the erupted tooth. With a given species of animal the radiophosphorus metabolism of the dental tissues decreases with age. Fujisaki, et al. (23), in a comparison of older rats (2.5 years) with younger ones (16-37 days) showed radiophosphorus incorporation into teeth of the older rats was one-half that of the younger rats.

Sobel and Lawrence (11,24) have shown that the carbonate to phosphate ratio of bones and teeth are related to the carbonate to phosphate ratio of blood serum. The carbonate to phosphate ratios in blood serum, dentin, enamel, and bone were highest for rats fed high calcium and low phosphate diets. They found that susceptibility to caries was about double in the teeth exhibiting high carbonate to phosphate ratios. This follows Sobel's theory (2) of increased caries

susceptibility with increased acid solubility of the tooth when the carbonate ratio is increased, and less caries susceptibility when the solubility of the enamel is decreased when the carbonate to phosphate ratio is lowered.

McClure (10) also demonstrated a striking inhibitory effect of disodium phosphate on experimental caries in the rat. On a wheat flour diet to which 1.5 per cent of disodium phosphate had been added, only 5.1 per cent of the rats in the experimental group had caries, and the average number of carious teeth per rat was 0.05. Control animals receiving a wheat flour diet with no disodium phosphate added averaged 2.97 carious teeth per rat, and 81.0 per cent of the rats had caries. When calcium phosphate was added to the wheat flour diet, no inhibitory effect on caries was noted. Also intubated disodium phosphate, which bypasses the oral cavity, is not as cariostatic as when the phosphate is consumed as part of a diet. The studies suggest strong evidence that the cariostatic effect of these phosphate minerals occurs within the oral cavity. It does not appear likely that the primary effect involves a systemic utilization of the phosphate supplement. The relative solubility of the phosphate appears to be an important factor.

Wynn, Haldi, Bentley, and Law (9) varied the calcium to phosphorus ratio in cariogenic diets. The calcium to phosphorus ratio in the diets were 1:0.5, 1:1, 1:2, and 1:3. The calcium content of all four diets was maintained at a constant level of 0.5 per cent of the entire diet. They found a progressive decrease in the caries score as the calcium to phosphorus ratio was decreased from 1:0.5 to 1:2. Further decrease in the ratio to 1:3 had no effect on the incidence or extent of caries.

Analysis of non-carious teeth showed that the nitrogen, calcium, phosphorus, magnesium, fluoride, and carbon dioxide content of the teeth was the same on each of the four diets. They felt this indicated that the effect of varying the calcium to phosphorus ratios of the diet on its cariogenicity was, therefore, not related to changes in composition of the teeth.

Wynn, Haldi, and Law (25) also found that if the phosphorus content of diets were held constant, and the calcium to phosphorus ratio was changed by adding calcium, the diet could likewise be made less cariogenic. These findings were taken to indicate that the changes induced in the cariogenic properties of their high sucrose diet, by altering the calcium and phosphorus content, were due to differences in the actual calcium and phosphorus content of the diets and not to differences in their calcium to phosphorus ratios. This was tested by increasing the calcium and phosphorus content of the diet without changing the calcium to phosphorus ratio. An increase in the calcium and phosphorus from 0.25 to 0.50 to 1.00 per cent gave a progressive decrease in the number of carious lesions and the caries score. The caries conductiveness of the diet was not affected by a further increase in the calcium and phosphorus concentration to 1.5 per cent. Thus their results show that not only is there a maximum level of anticariogenic activity for calcium and phosphorus, but the cariogenicity of the caries test diet is influenced by the actual amount of the calcium and phosphorus in the diet and not by the calcium to phosphorus ratio. This is in direct conflict with the findings of Sobel and Lawrence (11,24), which indicated a change in carbonate to phosphate ratio when the calcium to phosphorus

ratio was lowered in diets.

However, McClure (10) also found that the apparent gross ash, calcium, and phosphorus content of the enamel and dentin is not modified by a calcium or phosphorus supplement. He concluded that the phosphate minerals possibly act directly on the enamel surfaces of the teeth. Therefore, through a common ion effect, the dissolution of enamel or dentin may be reduced, thus limiting the caries decalcification process.

This is in partial agreement with Sobel, et al. (20), who hypothesize possible oral effects of high phosphate diets. They state that a change in the composition of saliva brought about by diets high in soluble phosphate can affect caries susceptibility in four possible ways. First, salivary phosphate may be increased via either systemic or direct oral effects. The elevated activity product $(A_{Ca^{++}})(A_{HPO_4})$ which would result would decrease dissolution of the tooth mineral, and, if the product was sufficiently high, it might actually be instrumental in remineralizing the partially dissolved surface of the enamel crystal. Second, by increasing salivary phosphate, the carbonate to phosphate ratio, as well as the citrate to phosphate ratio, is decreased, thus decreasing the solubility of the tooth mineral. Third, phosphate is a powerful buffer, and increased salivary phosphate, particularly by direct solution in the saliva or adsorption to the tooth surface during chewing, would neutralize bacterial acids. A fourth possible way in which increased salivary phosphate may decrease caries susceptibility may be by alteration of the bacterial flora present in the mouth.

Volker and Cremer (26) found that the calcium to phosphorus ratios for rat molars was 3.06 ± 0.54 for normal teeth and 2.44 ± 0.34

for carious teeth. However, a possible discrepancy would be the age of the teeth when examined. As stated before, the effect of serum levels is much greater on unerupted and young erupted teeth as compared to older erupted teeth. More important is the fact that the investigators who claim no difference in mineral content of carious and noncarious teeth may not recognize slight differences in mineral content. For example, Wynn, et al. (9), who state no difference in dentin when the calcium to phosphorus ratio in their diets were decreased, did not recognize a change in phosphorus content of 12.7 $\pm$ 0.1 per cent in 1:0.5 diet to 13.3 $\pm$ 0.1 per cent in a 1:3.0 diet. Nor did they recognize a decrease in nitrogen content from 2.85 $\pm$0.10 per cent to 2.78 $\pm$ 0.04 per cent in the same diets. Collidge (27) in his analyses of normal enamel and of normal enamel from early smooth surface caries qualifies his findings in that he states they show no large general differences in phosphorus or nitrogen content.

This leads to the importance of the measurement of nitrogen as a direct relation to the amount of organic material or protein found in teeth. Fellenberg and Schmidt (29) found that the organic substance of teeth consists mainly of proteins and small quantities of fat. They classified the proteins into glutin, collagen, and keratin. They also found that milk teeth, containing about 70 per cent glutin, are higher in protein than later teeth, that healthy teeth contain more of all three proteins than carious teeth, and that the protein content of teeth decreases with advancing age.

Coolidge (27) showed that there is a variation in the nitrogen and phosphorus content of enamel from different teeth and from different

areas of the same tooth. Coolidge (27) and Bhussary (29) found that the outermost areas of normal enamel show a higher nitrogen content in general than deeper layers. The study of amino acid content of human dentin (30,31) has revealed the presence of approximately twenty amino acids. The insoluble protein of dentin was found to be a collagen characterized by a high content of glycine, proline, and hydroxyproline. Human dentin averages 21-22 per cent organic matrix, of which 1-2 per cent is non-protein. The amino acids of decalcified and water extracted dentin accounted for 98.18 per cent and 97.01 per cent of the respective total nitrogen. The non-protein content was found to be mostly citric acid, aspartic acid, glutamic acid, mucoid, lipid, and cholesterol. From this it can be seen that a protein anabolic agent may have direct effects on protein content of teeth.

The problem of how to trace the effect of methandrostenolone on tooth structure was then considered. It was obvious that if protein was removed or protein formation increased, the amount of hydroxyapatite in teeth would also be affected. If the concentration of hydroxyapatite is altered, the concentration of phosphorus is also altered directly; thereby giving a source to trace the effects of methandrostenolone on tooth structure. If radioactive phosphorus is present in the blood serum, the increase or decrease of phosphorus metabolism by the teeth of the rats could be measured and related to this portion of tooth metabolism.

A study of Konig and Marthaler (32) on the eruption time of molars in experimental Sprague-Dawley rats showed that the first molars are completely erupted in seventeen days, the second molars in twenty days,

and the third molars in thirty-seven days. Thus by starting the experiment with weanling rats approximately twenty-five days old, it was also theoretically possible that we might be able to relate metabolism effects between erupted, newly erupted, and non-erupted teeth.

EXPERIMENTAL PROCEDURE

A total of 144 weanling Sprague-Dawley* strain albino rats, weighing from 45-50 grams each, was divided randomly into three groups of 24 females and 24 males. Males and females were housed on opposite sides of a portable battery of cages and placed three to a suspended screenwire botton cage. All animals were given distilled water adlibitum and supplemented weekly with oral doses of a concentrated vitamin A, D, and E mixture. All cages were washed and decontaminated once a week with soap and water. Feed cups were washed every morning. All animals were weighed twice weekly, fed fresh diet each day, and injected intraperitoneally with their respective radiophosphorus doses once a week. The radiophosphorus dose was two microcuries per one hundred grams of body weight. Solutions for injection were prepared from a stock solution of $H_3P^{32}O_4$ in weak hydrochloric acid by diluting a specified amount of isotope volumetrically with 0.001M sodium phosphate to a calculated volume. Thus, a stock solution was obtained for each injection time whereby no animal's required dosage was present in more than one milliliter of stock solution. Each stock solution contained a concentration of 1/10,000 Merthiolate as a bacteriostatic agent. The first injections were made on the first day of the experiment and injections were given every seven days thereafter.

The animals were given identical quantities of diet weighed on a K5T Kilo-Gramatic General-Purpose Balance. Variations of the basic

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* Sprague-Dawley Incorporated, Madison, Wisconsin.

caries-test diet (Stephan Diet 580*) were as follows:

Group 1. Animals were fed the basic diet to which was added two per cent disodium phosphate** and methandrostenolone.

Group 2. Animals were fed the basic diet to which was added two per cent disodium phosphate.

Group 3. Animals were fed the basic diet to which was added methandrostenolone.

Group 4. Animals were fed only the basic diet.

Those animals treated with methandrostenolone received a daily dose of approximately 5 mgm, per Kgm. of body weight during their experimental period. The drug was thoroughly incorporated into stock diets by the method of geometric dilution. The amount of drug incorporated was based on the average weight of the animals and their approximated weight gain between weighings. The precalculated aliquot of stock diet to be given in each feeder of the designated groups was based on the average quantity of food consumed per animal per day. This aliquot was substituted for an equal quantity of the group diet in each feeding cup and was placed on top of the basic diet to insure that the drug diet would be consumed. Each aliquot was large enough so that more than one sitting for each of the three rats in the cage would be required for its consumption. The following morning refused food was discarded after weighing. The method insured that each rat would receive its daily dosage and that all groups would receive the same amount of available diet.

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* Stephan 580 Caries-Test Diet: Skim Milk Power, 32%; Cane Sugar, 66%; Whole Dried Liver Substance N. F., 2%.

** Anhydrous Disodium Phosphate (2%) was substituted for 2% of the cane sugar content in the original caries-test diet.

Chemical analysis* of the caries test diet, with and without the disodium phosphate, is shown in Table 1. Each test was conducted in duplicate according to the Official Methods of Analysis of the Association of Official Agricultural Chemists. (33) An analysis of the caries test diets was obtained to determine the content of phosphorus in the basic diet as compared to that in the basic diet to which the two per cent disodium phosphate had been added. As may be seen in Table 1, the basic diet contained about half the phosphorus found in the basic diet supplemented with disodium phosphate. Thus, animals subsisting on basic diet received considerably less phosphorus than those animals fed the

Table 1. Chemical Analysis of the Caries-Test Diets (Average of Two Determinations)

Analysis	Basic Diet (Per Cent)	Basic Diet Plus Added Disodium Phosphate (Per Cent)
Moisture	1.27	1.21
Ether Extract	0.26	0.14
Crude Protein	12.47	12.38
Ash	2.92	4.60
Nitrogen-free extract	83.08	81.67
Phosphorus	0.36	0.70
Calcium	0.45	0.45
Sodium	0.25	0.97

basic diet with added disodium phosphate.

*Analysis conducted by South Dakota State College, Agricultural Experiment Station, Biochemistry Department.

At the end of two weeks, 48 animals (24 male and 24 female) were sacrificed with chloroform. Bilateral extractions of the maxillary and mandibalary molars were performed by elevating with scalers modified so that the tip was semipointed.

The upper jaw, left and right, first molar teeth were paired and analytically weighed with a Model H-5 Gram-atic Mettler Balance. The teeth were dissolved in approximately 1 ml. of nitric acid, with the aid of infrared heat lamps, in a pyrex glass planchet one inch in diameter and five-sixteenth inch in depth. This procedure was followed for upper jaw second and third molars, as well as for lower jaw first, second, and third molars. The samples were dried under infrared heat lamps and the radioactivity determined with three Tracerlab "1000" Scalers connected to shielded end-window Geiger tubes. All scalers were calibrated periodically to one selected as a standard by using a simulated P^{32} reference source under an aluminum absorber 138.75 mgm. per $cm.^2$ in thickness. All counts were corrected to the standard scaler by the periodic correction factors obtained by the calibrations. Each sample was counted five times, with sufficient time allowed to accumulate at least 500 counts per determination; thus, incurring a probable error of approximately three per cent.

Samples were corrected to time zero using the formula $N = N_o e^{-\lambda t}$, where N is the radioactivity at the time of determination, t; λ is the disintegration constant (1.96×10^{-3} hours) for P^{32}; e is the logarithm to the base e; and N_o is the radioactivity at time zero. Time zero was the time when the first sample of radioactivity was counted for the time interval under investigation.

After four weeks, 47 animals (23 male and 24 female) were sacrificed. Each group of animals was treated in the same manner as discussed above for those rats killed after two weeks of experimentation.

The sets of molars for each group and for each time period of experimentation were treated to a statistical analysis of their final counts per minute per mgm. of tooth. The following formulas were used to find:

1. Arithmetic mean ($\bar{X}$)

$$\bar{X} = \frac{\{ X_i}{N}$$

X_i = each individual measurement

N = number of individual measurements

$\{$ = sum

2. Standard Deviation (S)

$$S = \pm \sqrt{\frac{\{ (X_i - \bar{X})^2}{N-1}}$$

3. Significant difference between two means using the "T" test.

$$T = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}}$$

degrees of freedom = $(N_1 - 1) + (N_2 - 1)$

$\bar{X}_1$ = mean one

$\bar{X}_2$ = mean two

$\frac{S_1}{N_1}$ = standard deviation of mean one divided by number of individual measurements

$\frac{S_2}{N_2}$ = standard deviation of mean two divided by the number of individual measurements

Probability tables were then consulted using the "t" factors obtained for the calculated degrees of freedom. All significant differences in counts per minute per milligram of tooth were based on a probability of 0.05 or less ($P \leq 0.05$). This is to say that one may state with at least 95 per cent confidence that the counts per minute per milligram of tooth are significantly different with a probability equal to or less than 0.05.

RESULTS

The final average weights and the average weight gains for the groups of rats studied over each time interval were calculated. Examination of Table 2 shows that the final average weights and the average weight gains in each time interval of study are comparable to those found in the work of Shaw and Bailey (17,18). It is of interest to note that the female rats in groups 1 and 3 of the six-week time interval show a higher final average weight than other females and all males except the basic diet control (group 4). The final average drug dosages all approximate the desired dosage range of 5.0 mgm. per Kgm. body weight.

The average radioactivity in counts per minute per milligram of molar teeth, plus or minus standard deviation, was calculated for each group of animals. This data may be found in Tables 3 and 4.

The statistical comparisons of radio-phosphorus content between various groups were computed according to the method described on page 19. The results may be seen in Table 5. Although significant differences were based on a probability of 0.05, the majority of cases were significantly different at a probability level of 0.01.

Analysis of Table 5 shows that with only one exception, the radio-phosphorus content in molar teeth of animals fed the basic diet supplemented with methandrostenolone and disodium phosphate (group 1) was equal to that of the control animals fed the basic diet supplemented with disodium phosphate (group 2). The exception being the case in which the group 1 paired lower third molars of the male four-week

Table 2. Average Weight Gains, Final Average Weights, and Final Average Drug Dosages for Groups of Rats Studied Over Each Time Interval

Time Interval in Weeks	Group	Final Average Weight per Rat in Grams	Average Gain per Day per Rat in Grams	Final Average Drug Dosage mgm./Kgm.
		MALE		
2	1	64.7	1.36	5.37
	2	67.0	1.58	----
	3	72.3	1.88	5.01
	4	67.5	1.56	----
4	1	105.7	2.27	5.17
	2	102.7	2.04	----
	3	113.8	2.45	4.91
	4	101.3	2.11	----
6	1	125.5	2.06	5.57
	2	121.2	1.91	----
	3	127.2	2.12	5.61
	4	151.7	2.81	----
		FEMALE		
2	1	70.5	2.05	5.40
	2	72.5	2.05	----
	3	73.7	2.28	5.08
	4	71.0	1.96	----
4	1	104.7	2.26	5.07
	2	99.5	2.00	----
	3	103.2	2.19	5.22
	4	106.0	2.28	----
6	1	144.6	2.45	5.08
	2	126.2	2.23	----
	3	144.5	2.57	4.84
	4	132.2	2.33	----

Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
Group 2: 2% Na_2HPO_4 added to diet
Group 3: Methandrostenolone added to diet
Group 4: Basic diet

Table 3. Radio-phosphorus Located in the Molar Teeth of Male Rats

Animals	Group	Average Cpm.*/mg. ± Standard Deviation					
		Upper First	Upper Second	Upper Third	Lower First	Lower Second	Lower Third
		Two Week Interval					
6	1	15.4 ±2.22	15.4 ±2.86	41.6 ±4.40	15.5 ±4.42	15.8 ±2.43	42.1 ±3.78
6	2	12.6 ±3.93	13.2 ±3.74	39.9 ±10.1	12.8 ±2.85	13.5 ±3.66	40.2 ±9.53
6	3	23.6 ±4.60	21.3 ±6.15	59.5 ±13.8	20.5 ±4.90	21.1 ±4.64	56.4 ±4.14
6	4	21.5 ±4.87	21.1 ±5.71	67.9 ±6.83	22.8 ±1.11	20.9 ±4.70	62.7 ±4.45
		Four Week Interval					
6	1	13.3 ±0.97	12.7 ±1.37	27.7 ±5.82	13.3 ±0.71	13.0 ±1.97	23.8 ±7.42
6	2	13.1 ±1.14	12.6 ±1.64	27.6 ±3.71	13.6 ±1.34	13.1 ±1.44	27.5 ±2.42
6	3	22.4 ±1.64	20.3 ±2.08	38.9 ±7.18	23.2 ±2.22	21.5 ±1.93	43.0 ±4.74
6	4	21.0 ±2.28	20.4 ±2.55	42.8 ±5.06	22.7 ±1.23	20.7 ±1.97	43.1 ±4.27

Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
Group 2: 2% $NaHPO_4$ added to diet
Group 3: Methandrostenolone added to diet
Group 4: Basic Diet

*Cpm - counts per minute

Table 3. (Continued)

Animals	Group	Average Cpm.*/mg. ± Standard Deviation					
		Upper First	Upper Second	Upper Third	Lower First	Lower Second	Lower Third
		Six Week Interval					
6	1	16.0 ±1.62	14.4 ±1.68	23.7 ±2.04	16.0 ±3.01	16.5 ±1.94	24.7 ±2.67
5	2	15.0 ±0.74	14.4 ±1.96	23.5 ±1.88	15.1 ±0.86	14.8 ±1.35	24.5 ±1.17
6	3	24.1 ±1.53	19.2 ±3.93	35.6 ±5.78	25.7 ±3.87	25.4 ±8.47	35.8 ±8.15
6	4	24.1 ±2.13	22.6 ±0.97	34.8 ±3.49	22.3 ±4.41	25.2 ±5.26	35.8 ±3.39

* Cpm: Counts per minute

Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
Group 2: 2% $NaHPO_4$ added to diet
Group 3: Methandrostenolone added to diet
Group 4: Basic diet

Table 4. Radio-phosphorus Located in the Molar Teeth of Female Rats

Animals	Group	Average Cpm.*/mg. ± Standard Deviation: Upper First	Upper Second	Upper Third	Lower First	Lower Second	Lower Third
		Two Week Interval					
6	1	15.4 ±4.88	14.5 ±1.45	41.2 ±13.8	14.2 ±4.42	14.2 ±4.74	44.7 ±15.2
6	2	14.1 ±1.39	12.7 ±1.81	36.0 ±5.56	13.6 ±1.88	12.9 ±2.08	36.4 ±4.55
6	3	21.1 ±4.68	19.4 ±1.54	49.5 ±12.2	20.0 ±4.62	18.6 ±4.47	56.0 ±11.5
6	4	22.0 ±1.36	20.3 ±2.06	57.5 ±6.71	20.8 ±2.96	20.3 ±2.45	62.3 ±5.07
		Four Week Interval					
6	1	12.2 ±1.83	11.4 ±2.02	20.9 ±3.97	12.5 ±1.79	11.4 ±2.38	22.7 ±5.00
6	2	13.5 ±1.48	11.8 ±1.59	24.6 ±1.94	13.7 ±1.58	13.1 ±1.03	23.9 ±1.33
6	3	19.2 ±4.46	18.4 ±1.14	33.7 ±5.18	21.2 ±1.96	19.6 ±1.92	33.9 ±5.06
6	4	20.4 ±1.44	19.2 ±2.21	38.7 ±3.85	21.7 ±2.26	21.1 ±2.40	40.6 ±2.98

Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
Group 2: 2% $NaHPO_4$ added to diet
Group 3: Methandrostenolone added to diet
Group 4: Basic Diet

*Cpm = counts per minute

Table 4. (Continued)

Animals	Group	Upper First	Upper Second	Upper Third	Lower First	Lower Second	Lower Third
		Average Cpm.*/mg. ± Standard Deviation					
		Six Week Interval					
5	1	14.9 ±2.05	13.9 ±0.88	22.7 ±1.59	15.5 ±0.89	14.6 ±0.63	23.2 ±2.95
6	2	14.5 ±0.50	13.5 ±1.05	21.3 ±1.45	15.6 ±1.80	14.7 ±1.01	22.6 ±2.24
6	3	25.6 ±2.51	22.6 ±1.87	37.7 ±4.12	24.2 ±4.00	24.1 ±2.67	38.4 ±3.02
6	4	23.1 ±2.21	22.1 ±1.07	34.3 ±4.05	24.8 ±1.67	22.0 ±1.41	35.7 ±3.32

*Cpm = counts per minute

Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
Group 2: 2% $NaHPO_4$ added to diet
Group 3: Methandrostenolone added diet
Group 4: Basic Diet

Table 5. Statistical Relations* of Radio-phosphorus Content in Molar Teeth

Time Interval in Weeks	Paired Lower First Molar		Paired Upper First Molar	
	Male Relations	Female Relations	Male Relations	Female Relations
2	MP = P	MP = P	MP = P	MP = P
2	M = C	M = C	M = C	M = C
2	MP < M	MP < M	MP < M	MP < M
2	MP < C	MP < C	MP < C	MP < C
2	M > P	M > P	M > P	M > P
2	P < C	P < C	P < C	P < C
4	MP = P	MP = P	MP = P	MP = P
4	M = C	M = C	M = C	M = C
4	MP < M	MP < M	MP < M	MP < M
4	MP < C	MP < C	MP < C	MP < C
4	M > P	M > P	M > P	M > P
4	P < C	P < C	P < C	P < C
6	MP = P	MP = P	MP = P	MP = P
6	M = C	M = C	M = C	M = C
6	MP < M	MP < M	MP < M	MP < M
6	MP < C	MP < C	MP < C	MP < C
6	M > P	M > P	M > P	M > P
6	P < C	P < C	P < C	P < C

(MP) Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
(P) Group 2: 2% Na_2HPO_4 added to diet
(M) Group 3: Methandrostenolone added to diet
(C) Group 4: Basic diet

Significance: $P \leq 0.05$

Table 5. (Continued)

Time Interval in Weeks	Paired Lower Second Molar		Paired Upper Second Molar	
	Male Relations	Female Relations	Male Relations	Female Relations
2	MP = P	MP = P	MP = P	MP = P
2	M = C	M = C	M = C	M = C
2	MP < M	MP = M	MP < M	MP = M
2	MP = C	MP < C	MP < C	MP < C
2	M > P	M > P	M > P	M > P
2	P < C	P < C	P < C	P < C
4	MP = P	MP = P	MP = P	MP = P
4	M = C	M = C	MC = C	MC = C
4	MP < M	MP < M	MP < M	MP < M
4	MP < C	MP < C	MP < C	MP < C
4	M > P	M > P	M > P	M > P
4	P < C	P < C	P < C	P < C
6	MP = P	MP = P	MP = P	MP = P
6	M = C	M = C	M = C	M = C
6	MP < M	MP < M	MP < M	MP < M
6	MP < C	MP < C	MP < C	MP < C
6	M > P	M > P	M > P	M > P
6	P < C	P < C	P < C	P < C

(MP) Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
(P) Group 2: 2% Na_2HPO_4 added to diet
(M) Group 3: Methandrostenolone added to diet
(C) Group 4: Basic diet

Significance: $P \leq 0.05$

Table 5. (Continued)

Time Interval in Weeks	Paired Lower Third Molar		Paired Upper Third Molar	
	Male Relations	Female Relations	Male Relations	Female Relations
2	MP = P	MP = P	MP = P	MP = P
2	M < C	M = C	M = C	M = C
2	MP < M	MP = M	MP < M	MP = M
2	MP < C	MP < C	MP < C	MP < C
2	M > P	M > P	M > P	M > P
2	P < C	P < C	P < C	P < C
4	MP < P	MP = P	MP = P	MP = P
4	M = C	M < C	M = C	M = C
4	MP < M	MP < M	MP < M	MP < M
4	MP < C	MP < C	MP < C	MP < C
4	M > P	M > P	M > P	M > P
4	P < C	P < C	P < C	P < C
6	MP = P	MP = P	MP = P	MP = P
6	M = C	M = C	M = C	M = C
6	MP < M	MP < M	MP < M	MP < M
6	MP < C	MP < C	MP < C	MP < C
6	M > P	M > P	M > P	M > P
6	P < C	P < C	P < C	P < C

(MP) Group 1: Methandrostenolone plus 2% Na_2HPO_4 added to diet
(P) Group 2: 2% Na_2HPO_4 added to diet
(M) Group 3: Methandrostenolone added to diet
(C) Group 4: Basic diet

Significance: $P \leq 0.05$

time interval animals showed a lower radio-phosphate content than their respective control animals of group 2.

The radio-phosphorus content in the molar teeth of animals fed the basic diet supplemented with methandrostenolone (group 3) was equal to that of the control animals fed basic diet (group 4) with two exceptions. The two exceptions were the paired lower third molars of the animals in the male two-week and the female four-week time intervals. In both instances, the group 3 animals fed the basic diet supplemented with methandrostenolone exhibited lower radio-phosphorus content than their respective control animals of group 4, fed only the basic diet.

There were four exceptions to the fact that the radio-phosphorus content of the animals in group 1 was less than the radio-phosphorus content of the animals in group 3. All the exceptions occurred in the animals of the two-week time interval. These exceptions were the female paired lower second and third molars and the female paired upper second and third molars. In all four cases, the radio-phosphorus content was equal for both groups. Also, the male paired lower second molars of the two-week time interval animals was the only exception to the fact that the radio-phosphorus content of the group 3 animals was less than the radio-phosphorus content of the group 4 animals. This exception also exhibited equal radio-phosphorus content in each group.

Continued comparisons between the remaining two available possibilities (group 2 vs. group 3 and group 2 vs. group 4) showed no exceptions to the trend of radio-phosphorus content. In all cases, the radio-phosphorus content of group 3 animals was greater than the

radio-phosphorus content of group 2 animals, and the radio-phosphorus content was less for all group 2 animals than the radio-phosphorus content of all group 4 animals.

It was of particular interest to note that for all possible group comparisons of radio-phosphorus content in the paired lower and upper first molars, there were no deviations from the normal trend of radio-phosphorus content. From Table 3 and 4 it can be noted that the radio-phosphorus content per mgm. of tooth is comparatively equal for all of the paired first and second molars throughout the time intervals studied. However, the paired lower and upper third molars exhibit a higher degree of radio-phosphorus content which decreases with an increase in the time intervals studied.

As a general statement, it can be said that the animals fed diets containing disodium phosphate exhibited less radio-phosphorus content than those animals fed diets lacking disodium phosphate.

DISCUSSION

The results show that methandrostenolone exhibited definite anabolic effect on the female animals in this experiment which can only be seen clearly in the female animals of the six-week time interval. The lack of conclusive anabolic evidence of the animals in the two and four-week time intervals is probably due to the more rapid growth rates of all the animals before the six-week time interval. Because methandrostenolone is a derivative of the male hormone testosterone, it is interesting to find this occurance only in the female rats when non-gonadectomized rats were used over the short period of this study. The definite anabolic action in the six-week time interval does indicate that drug action was occurring during the experimentation period.

Because the third molars show a consistently higher radiophosphorus content than do the first or second molars, the findings of Bevelander and Amler (7) were verified. They also found that phosphate exchange was greater for unerupted teeth than for the older and mature teeth.

However, the findings of Sobel and Hanok (4) that dietary phosphate composition affects blood phosphate composition, which in turn affects tooth phosphate composition, cannot be completely verified by the results of this experiment. It appears that the increased dietary amount of phosphate was not accompanied by an increase in phosphate content of the teeth. The results conversely show a consistency of lower radiophosphorus content whenever disodium phosphate was added to the basic diet. To attribute this phenomena to the dilution of radiophosphorus by the rise

of phosphate concentration in the blood does not appear to be outside the realm of feasibility.

The results also show that animals fed methandrostenolone-supplemented diets exhibited no increase of radio-phosphorus content over the teeth of animals fed only the basic diet. Assuming that the addition of disodium phosphate to the basic diets exerted cariostatic action on the animals that were fed these diets, it appears that methandrostenolone did not contribute to any increased utilization of the phosphate during the various stages of phosphate metabolism. This would be in agreement with McClure's (12) findings that it does not appear likely that the primary effect of disodium phosphate as a cariostatic agent envolves a systemic utilization of the phosphate supplement. Because the radio-phosphorus was injected into all rats, an accurate determination cannot be made of McClure's suggestion that there would be increased adsorption of phosphate on the exterior portion of the teeth of the animals in this study.

Because the results show no trend towards the increase of phosphate metabolism in the molars of the animals fed diets containing methandrostenolone with or without disodium phosphate, the hypothesis that an anabolic agent would increase the formation of hydroxyapatite was not illustrated to be true. These were also the findings of Shaw and Bailey (18). However, in their study of the effect of norethandrolone on dental caries in the rat (17), they found that norethandrolone increased caries in rat molars. It is possible that the exceptions in the comparison between group 1 and group 3 in Table 5 can now be explained on the basis of their results. Although the other

exceptions illustrated in the results are probably due to unusual metabolic differences or experimental error, the cited exceptions between groups 1 and 3 may be of extreme importance in this study.

The exceptions are in the two-week time interval for female paired lower second and third molars and the female paired upper second and third molars. The special importance of these exceptions are:

1. They occur in the time interval in which the third molars have just erupted (32). In addition, the second molars erupted approximately at the beginning of the time interval. It is during this period in the study that both the second and third molars have their highest metabolic rates.

2. If methandrostenolone is going to effect the phosphate metabolism of these molars, the optimum time is during this period of the study.

3. All four are exceptions to the trend that the radio-phosphorus content of the molars of rats, which have been fed diets supplemented with disodium phosphate is less than for those rats fed a diet containing no disodium phosphate.

4. Both comparisons of group 1 against group 4 and group 3 against group 2 have a probability of 0.05, which is on the borderline of not being considered significantly different.

5. It is known that methandrostenolone induces calcium utilization in the bones of the body. (19)

Considering all of these facts as a unit, there might be the possibility that incomplete hydroxyapatite is being formed during this period due to a lack of calcium. If this is so, not only is the protein matrix left vulnerable to bacterial proteolysis, but the solubility of this portion of the tooth is increased. Both of these factors would contribute to an increased susceptibility to the complete caries process. Following the formation of caries in these teeth during the next two-week time interval, there would be a reduction of phosphate which would appear in

the results as a lack of phosphate metabolism or as a higher caries score. A future study with methandrostenolone using a higher calcium content in the diets could possibly show if this possibility exists as Wynn, et al. (25), have already shown that the altering of both calcium and phosphorus content in caries-test diets produces cariostatic activity.

CONCLUSIONS

1. There was no statistical difference between the radio-phosphorus content of those animals fed basic diet supplemented with methandrostenolone as compared to the control animals fed only the basic diet.

2. There was no statistical difference between the radio-phosphorus content of those animals fed basic diet supplemented with methandrostenolone and disodium phosphate as compared to the control animals fed the basic diet supplemented with disodium phosphate.

3. The radio-phosphorus content of the molars of all animals fed a basic diet supplemented with disodium phosphate exhibited lower values than did the molars of those animals fed basic diets with no disodium phosphate added as a supplement.

4. Methandrostenolone exhibited no changing effect on the radio-phosphorus uptake in the molars of rats, with or without dietary phosphorus, over the time intervals used in this study.

5. Further studies should be made using diets containing higher calcium levels in conjunction with the administration of methandrostenolone and disodium phosphate.

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